

VISIT...

LANZAROTE
Caliente.COM

METABOLISM OF 18-METHOXYCORONARIDINE, AN IBOGAINE ANALOG, TO 18-HYDROXYCORONARIDINE BY GENETICALLY VARIABLE CYP2C19

WENJIANG ZHANG, YAMINI RAMAMOORTHY, RACHEL F. TYNDALE, STANLEY D. GLICK, ISABELLE M. MAISONNEUVE, MARTIN E. KUEHNE, AND EDWARD M. SELLERS

Center for Addiction and Mental Health (W.Z., R.F.T., E.M.S.), the Departments of Pharmacology (Y.R., R.F.T., E.M.S.), Medicine (E.M.S.), and Psychiatry (E.M.S.), University of Toronto, Toronto, Canada; Sunnybrook and Women's Health Science Centre, Toronto, Canada (E.M.S.); Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, New York (S.D.G., I.M.M.); and Department of Chemistry, University of Vermont, Burlington, Vermont (M.E.K.)

(Received September 10, 2001; accepted February 26, 2002)

This article is available online at <http://dmd.aspetjournals.org>

ABSTRACT:

18-Methoxycoronaridine, a newly developed ibogaine analog, has been reported to decrease the self-administration of morphine, cocaine, ethanol, and nicotine. It has also been reported to attenuate naltrexone-precipitated signs of morphine withdrawal. In this study, three metabolites of 18-methoxycoronaridine (18-MC) were separated and identified by high-performance liquid chromatography-electrospray ionization-mass spectrometry-mass spectrometry (HPLC-ESI-MS-MS); the major metabolite was 18-hydroxycoronaridine (18-HC). The other two metabolites were elucidated as hydroxylated metabolites on the basis of their MS-MS spectra. Catalytic studies of 18-MC O-demethylase activity in human liver microsomes indicate that one high affinity enzyme is involved in this reaction (K_m from 2.81 to 7.9 μM ; $V_{\max}$ from 0.045 to 0.29 nmol/mg/min). In cDNA-expressing microsomes, only CYP2C19 displayed significant 18-MC O-demethylase activity (K_m 1.34 μM ;

$V_{\max}$ 0.21 nmol/mg/min). S-Mephenytoin, a selective CYP2C19 inhibitor, inhibited 18-MC O-demethylation by 65% at a concentration of 2 times its K_i , and antibodies against rat 2C (human CYP2C8, 2C9, 2C19) inhibited 18-HC formation by 70%. Studies with other cytochrome P450 (P450)-selective chemical inhibitors and antibodies failed to demonstrate an appreciable role for other P450s in this reaction. In addition, in microsomes from five different human livers, 18-MC O-demethylation correlated with S-mephenytoin 4'-hydroxylase activity but not with other P450 probe reactions. These data indicate that 18-HC formation is the predominant pathway of 18-MC metabolism in vitro in human liver microsomes and that this metabolic pathway is primarily catalyzed by the polymorphic CYP2C19. The apparent selectivity of this pathway for CYP2C19 suggests 18-MC as a potentially useful probe of CYP2C19 activity in vitro and in vivo.

Ibogaine, an iboga alkaloid extracted from the root bark of the African shrub *Tabernanthe iboga*, has been observed to be effective in the treatment of opiate addiction and stimulant abuse (Alper et al., 1999; Mash et al., 2000). 18-Methoxycoronaridine (18-MC¹) (Fig. 1), a newly developed ibogaine analog, has been reported to decrease the self-administration of morphine, cocaine, ethanol, and nicotine, and to attenuate naltrexone-induced morphine withdrawal in rat models (Glick et al., 1996, 1998, 1999; Maisonneuve and Glick, 1999). In addition to having stimulant and hallucinogenic properties, ibogaine induces tremors and, at high doses, damages the cerebellum (Dzolic

et al., 1988; Glick et al., 1991, 1992, 1994; Cappendijk and Dzolic, 1993; O'Hearn and Molliver, 1993; Serksen et al., 1994). 18-MC, therefore, requires further study as a potentially useful therapeutic agent in the treatment of opiate and psychostimulant addiction, as well as for opiate withdrawal. Prior to beginning clinical trials, it is necessary to identify the metabolites of 18-MC, as well as the enzymes involved in its metabolism.

Cytochrome P450s (P450s) play a major role in the metabolism of endogenous and exogenous compounds. Over 20 P450 isoforms have now been characterized, eight of which have been shown to be the major enzymes involved in the oxidative metabolism of drugs (CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4) (Shimada et al., 1994). The polymorphic human isoform CYP2C19 contributes to the metabolism of many therapeutically important drugs (Michalets, 1998). The poor metabolizer (PM) phenotype, constituting those homozygous for inactive alleles and therefore having no CYP2C19 activity, occurs with a frequency of 2.5 to 6% in Caucasians. Higher frequencies are observed in Chinese (15–17%) and Japanese (18–23%) populations (Inaba et al., 1984; Wedlund et al., 1984; Jurima et al., 1985; Nakamura et al., 1985; Horai et al., 1989; Bertilsson et al., 1992). PMs may be more likely to experience adverse drug effects due to increased plasma concentration and/or increased half-life of the parent drug. For drugs that require CYP2C19-catalyzed bioactivation

Supported in part by National Cancer Institute Grant CA-12010 and National Institute on Drug Abuse Grants DA-03817 and DA-06889.

¹ Abbreviations used are: 18-MC, 18-methoxycoronaridine; P450, cytochrome P450; PM, poor metabolizer; HPLC, high-performance liquid chromatography; 18-HC, 18-hydroxycoronaridine; ESI, electrospray ionization; MS, mass spectrometry; α -NP, α -naphthoflavone; PL, pilocarpine; OP, orphenadrine; SP, sulfaphenazole; S-MP, S-mephenytoin; BD, budipine; DDC, diethylthiocarbamate; KT, ketoconazole.

Address correspondence to: Dr. Edward M. Sellers, Psychopharmacology and Dependence Research Unit, Sunnybrook and Women's College Health Science Center, Room 942, 76 Grenville St., Toronto, ON M5S 1B2 Canada. E-mail: e.sellers@utoronto.ca

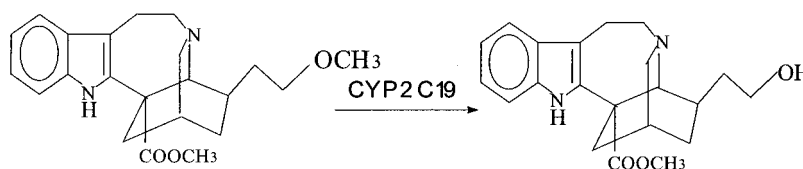


FIG. 1. Metabolism of 18-MC to 18-HC.

to a pharmacologically active metabolite, drug efficacy can be reduced in PMs.

In the present study, HPLC-MS/MS-MS was used to identify a major metabolite of 18-MC, 18-hydroxycoronaridine (18-HC). Moreover, using a variety of *in vitro* techniques, including the use of cDNA-expressing microsomes and P450-selective chemical and antibody inhibitors, the main P450(s) responsible for the formation of 18-HC were determined.

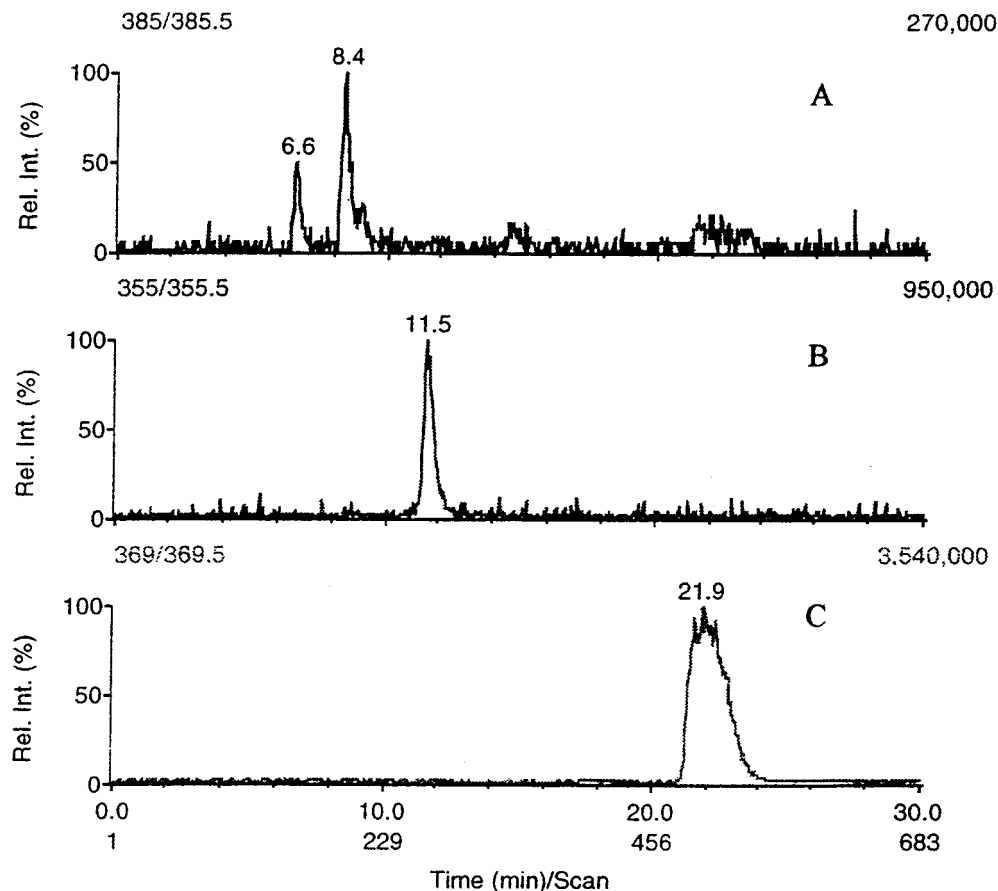
Experimental Procedures

Reagent and Biological Materials. 18-MC and 18-hydroxycoronaridine (18-HC) were kindly provided by Dr. Stanley Glick (Albany Medical College, Albany, NY) and Dr. Martin Kuehne (University of Vermont, Burlington, VT). Commercial sources were as follows. α -Naphthoflavone, pilocarpine, orphenadrine, sulfaphenazole, S-(+)-mephénytoin, budipine, diethyldithiocarbamate, ketoconazole, NADPH, and butorphanol were purchased from Sigma-Aldrich (St. Louis, MO). Human liver microsomes (HG03, HG30, HG43, HG90, HG161) and microsomes from human lymphoblast cells expressing CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1 or 3A4 were purchased from Gentest Corp. (Woburn, MA). Human liver microsomes from liver specimen L62 were prepared according to standard procedures. Monoclonal antibodies against human CYP2A6, 2B6, 2D6, and 2E1 were purchased from

Gentest Corp., whereas rat CYP2C13 (human CYP2C8/2C9/2C19 selective) and CYP3A2 (human CYP3A4 and CYP3A5 selective) polyclonal antibodies were from Daiichi Pure Chemical Co. Ltd. (Tokyo, Japan). All other chemical reagents used were of the highest commercially available quality.

HPLC-ESI-MS-MS Identification of 18-MC Metabolites. Incubation mixtures contained human liver microsomes (0.25 mg/ml), 18-MC (50 μ M), and NADPH (1.0 mM) in a total volume of 0.25 ml of Tris-HCl buffer (25 mM, pH 7.5). The reactions were initiated by the addition of NADPH and were incubated at 37 $^{\circ}$ C for 30 min. After extraction with 2 ml of ethyl acetate, the organic phase was evaporated to dryness and reconstituted into 200 μ l of acetonitrile and water (1:3). 18-MC and its metabolites were separated and identified by HPLC-ESI-MS-MS [Waters Spherisorb 5SC6 (5 μ M, 4.6 $\times$ 150 mm) column; 10 mM ammonium acetate (adjusted to pH 3.7 with acetic acid) in 25% acetonitrile at 1 ml/min]. The effluent was introduced into the ion spray interface of a PE Sciex API III⁺ mass spectrometer (PerkinElmerSciex Instruments, Boston, MA) operated in position ion mode; the source temperature was 500 $^{\circ}$ C, and the orifice voltage was 35V. Argon was used as the collision gas for collision induced dissociation (CID) at a thickness of 1.3×10^{14} atoms/cm². The collision energy was 45eV. CID required ion monitoring of *m/z* 385, 385, 355, and 369 at 6.6, 8.4, 11.5, and 21.9 min.

Assay of 18-MC O-Demethylase Activity. 18-MC O-demethylation was determined after incubating 18-MC concentrations (0.3–60 μ M) in human liver microsomes at the conditions listed above. Butorphanol (50 μ l of 5

FIG. 2. Selected ion chromatographs of 18-MC incubation in human liver (L-62) microsomes using HPLC-MS (A) *m/z* 385 (B) *m/z* 355 (C) *m/z* 369.

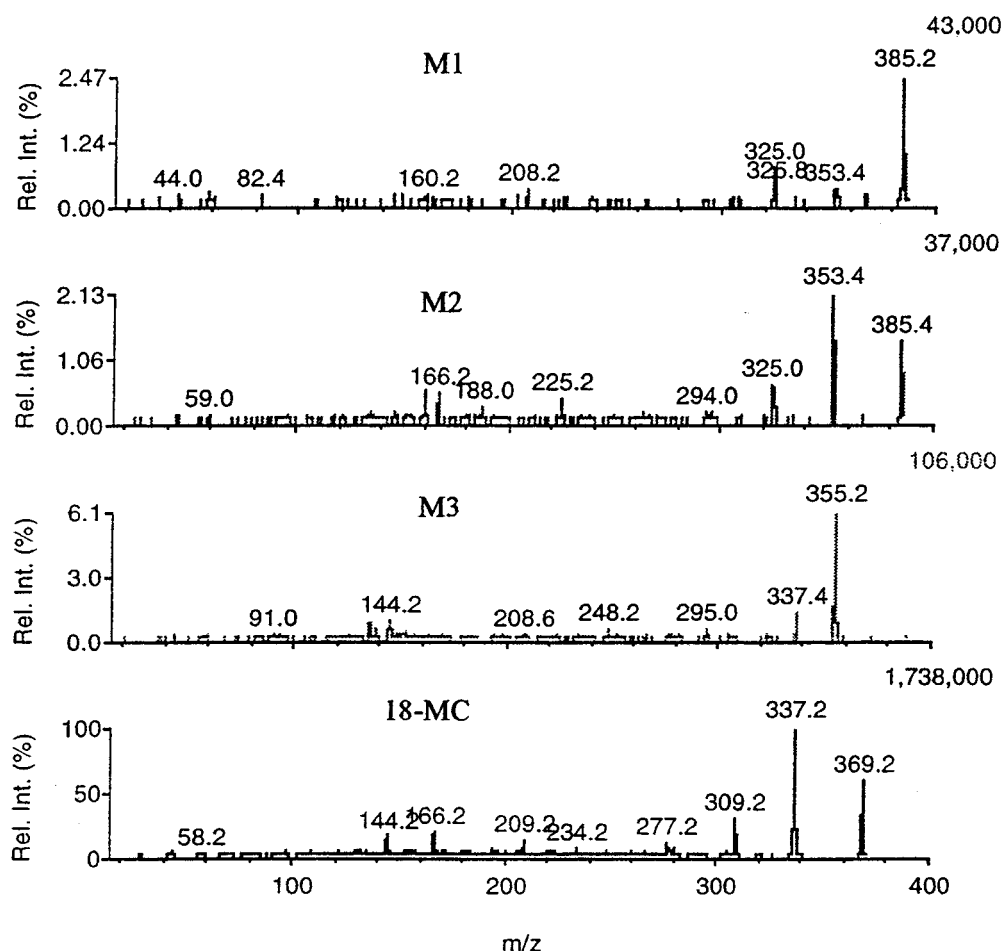


FIG. 3. MS-MS spectra of 18-MC and its metabolites.

18-MC (250 μ M) was incubated in human liver (L-62) microsomes for 30 min and analyzed by HPLC-ESI-MS-MS. The MS-MS spectra of M1, M2, M3, and 18-MC were obtained by CID of m/z 385, 385, 355, and 369 at 6.6, 8.4, 11.5 and 21.9 min, respectively.

μ g/ml) was added as the internal standard and the mixture was extracted with 2 ml of ethyl acetate. The organic phase was evaporated to dryness and reconstituted into 200 μ l of 25% acetonitrile prior injection into the HPLC column (Waters 5SC6, 5 μ m, 125 $\times$ 4.6 mm; Waters Corp.), UV = 220 nm, and acetonitrile: 10 mM potassium phosphate (pH 3.7) buffer (25:75, v/v) at 1 ml/min. 18-HC was quantified by interpolating the peak area ratio of 18-HC and butorphanol from a standard curve of known 18-HC concentrations. Incubations using cDNA expressing microsomes were conducted in a similar manner except that microsomal protein concentrations were adjusted for each expressed isoform to account for differences in expression level. Assays were linear with respect to time and protein concentration across the 18-MC concentration range used.

Chemical Inhibition. The selective inhibitors of eight major P450s (CYP1A1/1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4) were chosen according to the results of previous studies (Bourrie et al., 1996; Eagling et al., 1998): α -naphthoflavone (α -NP) (CYP1A1/1A2, K_i 0.01 μ M), pilocarpine (PL) (CYP2A6, K_i 4 μ M), orphenadrine (OP) (CYP2B6, K_i 4 μ M), sulfaphenazole (SP) (CYP2C9, K_i 0.3 μ M), S-(+)-mephenytoin (S-MP) (CYP2C19, K_i 175 μ M), budipine (BD) (CYP2D6, K_i 0.4 μ M), diethyldithiocarbamate (DDC) (CYP2E1, K_i 7.5 μ M), and ketoconazole (KT) (CYP3A4, K_i 0.015 μ M). Two concentrations of inhibitor were used: α -NP (0.5 μ M, 5 μ M), PL (10 μ M, 100 μ M), OP (10 μ M, 100 μ M), SP (0.5 μ M, 5 μ M), S-MP (200 μ M, 400 μ M), BD (0.5 μ M, 5 μ M), DDC (10 μ M, 100 μ M), and KT (0.5 μ M, 5 μ M). The substrate concentration was equal to the K_m derived from the human liver microsomes. In the case of inhibitors that have previously demonstrated mechanism-based inactivation of P450s (OP and DDC), the inhibitor was preincubated with microsomes and NADPH at 37 $^{\circ}$ C for 30 min prior to

the addition of the substrate. Control reactions for OP and DDC experiments included preincubation without inhibitor.

Immunochemical Inhibition. Immunoinhibition experiments were carried out in pooled human liver microsomes (HG161). Antibodies were preincubated with liver microsomes on ice for 15 min followed by the addition of 18-MC (at K_m concentration), 1 mM NADPH, and 25 mM Tris-HCl buffer. Antibody concentrations were chosen based on experiments performed by the manufacturers that showed concentration-dependent inhibition of probe P450 reactions.

Data Analysis. Metabolic constants for the kinetic data K_m and V_{max} were determined by nonlinear regression analyses after Michaelis-Menten transformation (rate of metabolite formation as a function of substrate concentration) with EnzPack 3 software (EnzPack, Cambridge, UK).

Results

Identification of 18-MC Metabolites. Following incubation of 18-MC with human liver microsomes, the extracted phase was separated by HPLC-ESI-MS and HPLC-ESI-MS-MS, and the resulting peaks interpreted. Figures 2A to C are selected ion chromatographs of m/z 385, 355, and 369, respectively. By comparison with the blank (incubation without 18-MC), the peaks at 6.6, 8.4, and 11.5 min can be identified as metabolites (M1, M2, and M3) of 18-MC. In Fig. 3, the MS-MS spectra of 18-MC, M1, M2, and M3 are compared. As illustrated, the molecular ion peak ($M + H$, m/z 369) of 18-MC is 14 mass units higher than the molecular ion peak ($M + H$, m/z 355) of M3. This suggests that M3 is produced by a demethylation of 18-MC ($-\text{OCH}_3$ versus $-\text{OH}$). As can be seen in Fig. 1, 18-MC demethyl-

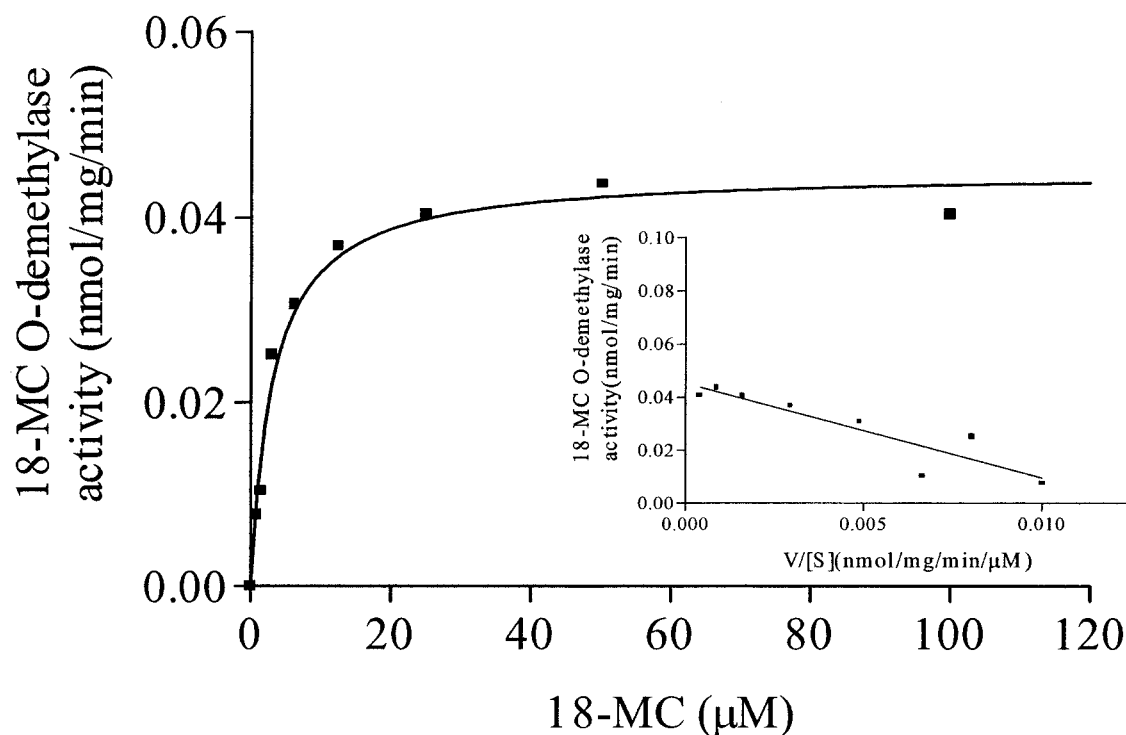


FIG. 4. Substrate saturation plot of 18-MC O-demethylation in human liver (HG161) microsomes with inset Eadie-Hofstee plot demonstrating monophasic kinetics.

ation may only occur at $-\text{COCH}_3$ or $-\text{COOCH}_3$. The loss of 18 (m/z 337, $\text{M}-\text{H}_2\text{O}$) and 60 mass units (m/z 295, $\text{M}-\text{HCOOCH}_3$) rather than formic acid (HCOOH) indicates that M3 is produced by demethylation at $-\text{COCH}_3$. This was confirmed by the MS-MS spectrum of the synthesized compound 18-HC, which has the same characteristic fragments as M3 (m/z 355, 337, 295, 277, 209, 144, and 135).

Figure 3 also demonstrates that the molecular ion peak of M1 ($\text{M} + \text{H}$, m/z 385) and M2 ($\text{M} + \text{H}$, m/z 385) are 16 mass units higher than the molecular ion peak of 18-MC ($\text{M} + \text{H}$, m/z 369). M1 and M2, therefore, are clearly two hydroxylated metabolites of 18-MC ($-\text{OH}$ versus $-\text{H}$). However, NMR or other similar methods are needed to confirm at which carbon the hydroxylation occurs for each metabolite.

18-MC O-Demethylase Activity in Human Liver Microsomes. Substrate saturation experiments conducted in pooled human liver microsomes demonstrated single-site kinetics, as demonstrated by the inset Eadie-Hofstee plot (Fig. 4). The apparent K_m and $V_{\max}$ values of 18-HC formation along with intrinsic clearances ($V_{\max}/K_m$) from six human liver microsomes, compared with cDNA-expressing CYP2C19 from human lymphoblast cells, are summarized in Table 1. Average K_m , $V_{\max}$, and $V_{\max}/K_m$ values with standard deviations were $4.5 \pm 1.9 \mu\text{M}$, $0.012 \pm 0.098 \text{ nmol/mg/min}$, and 0.19 ± 0.035 , respectively, for human liver microsomes. As can be seen, significant interindividual variability was observed, particularly in $V_{\max}$. Indeed, the intrinsic clearance of HG43 is more than 10 times higher than the intrinsic clearance of HG03.

P450 Screening in cDNA-Expressing Microsomes. To evaluate the contribution of the major P450s to the demethylation of 18-MC, two different concentrations of 18-MC (K_m and $10 K_m$ from human liver microsomes) were incubated with a series of cDNA-expressing microsomes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) from human lymphoblast cells. As shown in Fig. 5, of all the P450s tested, CYP2C19 displayed the greatest demethylation activity, whereas little activity was observed with CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, and 3A4. The apparent K_m and $V_{\max}$ values of 18-HC

formation in CYP2C19-expressing microsomes were $1.3 \mu\text{M}$ and 0.21 nmol/mg/min , respectively, similar to the values obtained in human liver microsomes. The relative roles of these P450s in the formation of the two unknown metabolites, M1 and M2, were also evaluated in cDNA-expressing microsomes. In contrast to 18-HC formation, CYP3A4 appeared to be the main enzyme responsible for M2 formation at the concentrations used, with CYP2C9 and 1A2 showing lower activity. CYP3A4 also showed the greatest M1 formation, although the other P450s examined also demonstrated appreciable activity at similar levels.

Inhibition of 18-HC Formation by P450-Selective Chemical Inhibitors. Selective inhibitors of the eight major P450s were used to identify the dominant P450s that mediate the formation of 18-HC in human liver microsomes (Fig. 6). S-(+)-MP (CYP2C19) inhibited the formation of 18-HC by 43 and 64% at concentrations of approximately K_i ($200 \mu\text{M}$) and $2K_i$ ($400 \mu\text{M}$), respectively. In contrast, KT (3A4), BD (2D6), and α -NP (1A1/1A2) did not inhibit the formation of 18-HC at more than 10 times their K_i concentrations. SP (2C9), PL

TABLE 1
Kinetic parameters of 18-MC O-demethylase activity in human liver microsomes and cDNA expressed CYP2C19

Human liver HG03, HG30, HG43, HG93, and HG161 microsomes and cDNA-expressing CYP2C19 from human lymphoblast cells were purchased from Gentest Corp. Human liver L-62 microsomes were prepared according to standard procedures.

Human Liver Microsomes	K_m	$V_{\max}$	$V_{\max}/K_m$
	μM	nmol/mg/min	
HG03	7.8	0.056	0.0072
HG30	5.2	0.18	0.034
HG43	2.8	0.29	0.10
HG93	3.3	0.050	0.015
HG161 (pooled)	4.9	0.084	0.017
L-62	3.2	0.045	0.014
Average $\pm$ SD	4.5 ± 1.9	0.012 ± 0.098	0.19 ± 0.035
CYP2C19	1.3	0.21	0.16

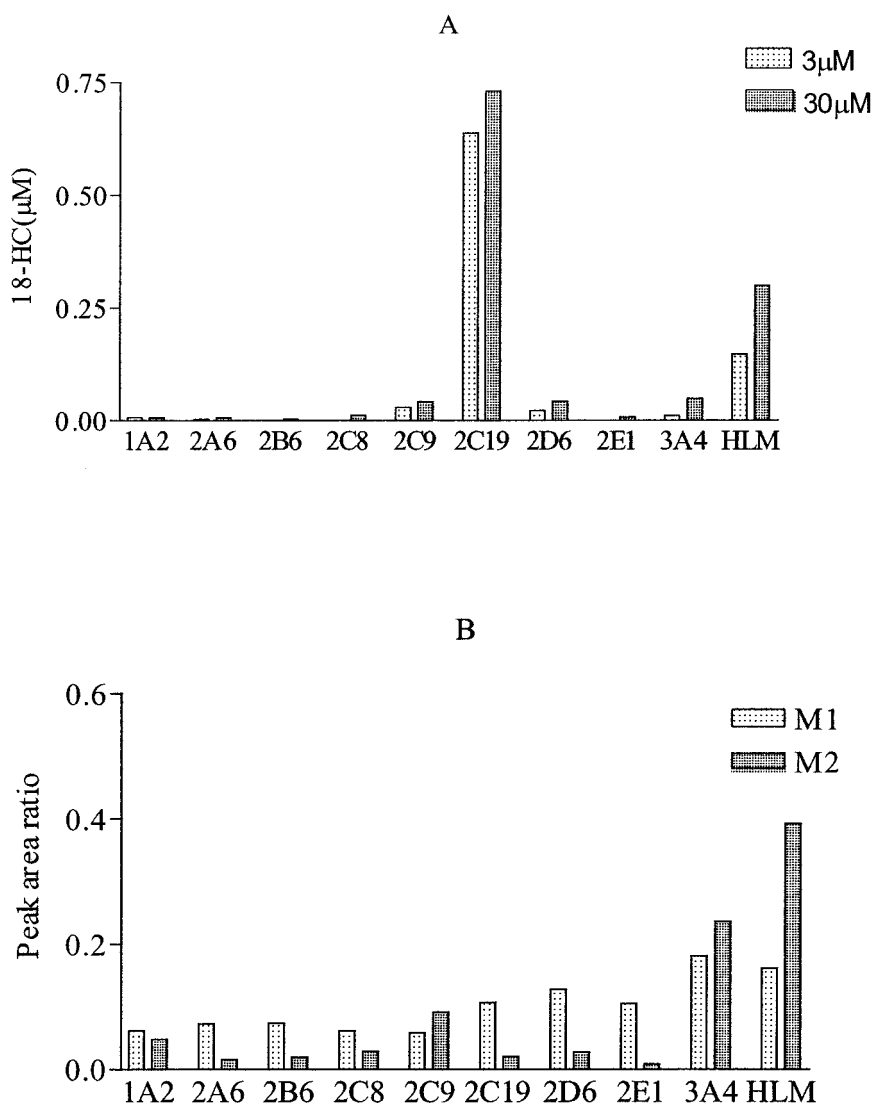


FIG. 5. A, 18-HC formation after incubation of 3 and 30 μ M of 18-MC in cDNA expressing P450s from human lymphoblast cells; B, peak area ratios (ratios of M1 and M2 to internal standard) after incubation of 30 μ M 18-MC in cDNA expressing P450s from human lymphoblast cells.

(2A6), and OP (2B6) were not able to inhibit the formation of 18-HC at their approximate K_i concentrations and were able to do so by only approximately 10% at more than 10 times their K_i concentrations. DDC (2E1) inhibited the formation of 18-HC at concentrations of K_i and $10K_i$ by 25 and 50%, respectively.

Immunoinhibition. The metabolic conversion of 18-MC to 18-HC in pooled human liver microsomes was inhibited by polyclonal antibodies against rat CYP2C13 (which reacts with human liver CYP2C8, 2C9, and 2C19) by approximately 70% (Fig. 7). CYP2A6-selective antibodies inhibited 18-HC formation by approximately 30%. Antibodies against CYP3A4/5, 2E1, 2D6, and 2B6 did not appear to inhibit 18-HC formation in human liver microsomes.

Correlation of Activity in Human Liver Panel. 18-MC O-demethylation activity, measured in human liver microsomes from five human livers, ranged from 0.050 to 0.29 nmol/mg/min. Correlations of these activities of human livers HG03, HG30, HG43, HG90, and HG161 with their standard P450 isoform-specific activities (as reported by Gentest Corp.) demonstrated a correlation only with CYP2C19 catalyzed S-mephenytoin 4'-hydroxylation ($r^2 = 0.97$) (Fig. 8).

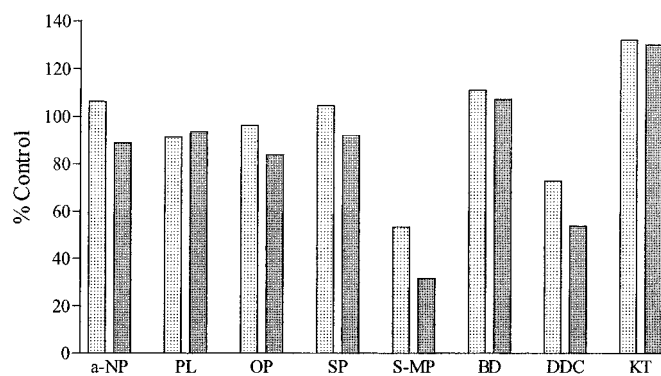


FIG. 6. Chemical inhibition of 18-HC formation from 18-MC in human liver microsomes.

Chemical inhibitors of P450s were added to the incubation at two different concentrations: α -naphthoflavone (α -NP, K_i 0.01 μ M) (0.5 μ M, 5 μ M), S-mephenytoin (S-MP, K_i 175 μ M) (200 μ M, 400 μ M), pilocarpine (PL, K_i 4 μ M) (10 μ M, 100 μ M), orphenadrine (OP, K_i 4 μ M) (10 μ M, 100 μ M), sulfaphenazole (SP, K_i 0.3 μ M) (0.5 μ M, 5 μ M), budipine (BD, K_i 0.4 μ M) (0.5 μ M, 5 μ M), diethyldithiocarbamate (DDC, K_i 7.5 μ M) (10 μ M, 100 μ M), and ketoconazole (KT, 0.015 μ M) (0.5 μ M, 5 μ M).

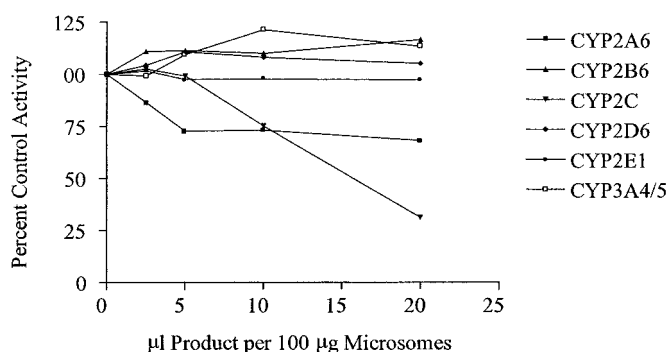


FIG. 7. Inhibition of 18-HC formation from 18-MC by P450-selective antibodies in human liver microsomes.

Antibody concentrations were chosen based on experiments performed by the manufacturers as stated under *Experimental Procedures*. For CYP2A6, CYP2B6, CYP2E1, and CYP2D6, they were 0, 2.5 μ l, 5 μ l, 10 μ l, and 20 μ l; for CYP2C and CYP3A4/5, they were 0, 5 μ l, 10 μ l, 20 μ l, and 40 μ l.

Discussion

We have identified three metabolites of the ibogaine analog 18-MC, which may have utility in the treatment of drug dependence, by HPLC-ESI-MS-MS. We have also determined that the *O*-demethylated metabolite, 18-HC, is produced mainly by CYP2C19.

HPLC-ESI-MS/MS-MS combines the high separation capacity of HPLC and the identification capacity of MS/MS-MS. It is advantageous over gas chromatography-MS in its ability to analyze unstable and highly polar drugs and in providing useful information about the structures of the drug and its metabolites. In this study, the main fragments of the 18-MC were first analyzed; *m/z* 144 and 160 were found to be its two characteristic ions. The two daughter ions were used to find the trace metabolites on the basis of the parent-daughter relationship by CID. Three metabolites were separated and identified; one was identified as 18-HC by MS-MS and then confirmed by the use of synthetic 18-HC. Two other metabolites (M1, M2) were determined to be the hydroxylated metabolites on the basis of their MS-MS spectra. These two metabolites, however, still require confirmation by the synthetic standard compound or NMR analysis. Moreover, a lower concentration of 18-MC should be used to better evaluate the role of P450s in the formation of these metabolites at more physiological concentrations.

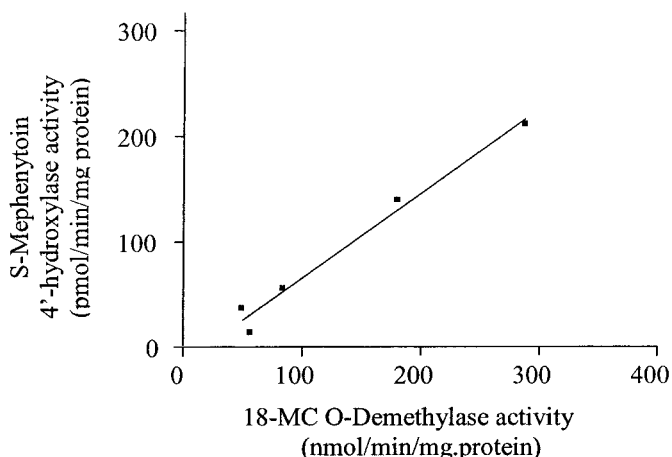


FIG. 8. Correlation between S-mephenytoin 4'-hydroxylase activity and 18-MC O-demethylase activity in human liver microsomes.

The data for S-mephenytoin 4'-hydroxylase activity in human livers HG003, HG030, HG043, HG093, and HG161 were as determined by Gentest Corp.

Numerous approaches have been developed for the identification of human cytochrome P450 isoforms involved in the metabolism of xenobiotics in vitro. A combined approach provides a high degree of certainty for the identification of the enzymes responsible for a specific metabolic pathway of a drug. In this study, chemical inhibitors of P450s, antibodies directed against P450s, and cDNA-expressing P450s were used to identify the enzymes responsible for 18-HC formation in human liver microsomes. As shown in Fig. 5, only CYP2C19 produced significant amounts of 18-HC in screening experiments in cDNA-expressing microsomes. Little to no metabolic activity was observed with CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, and 3A4. Moreover, K_m and V_{max} values of 18-MC in cDNA-expressing CYP2C19 were similar to those obtained in human liver microsomes. In addition, S-MP, a CYP2C19 selective inhibitor, blocked the formation of 18-HC by approximately 43 and 64% at concentrations of K_i and $2K_i$, respectively. Antibodies against rat CYP2C13 (human 2C8, 2C9, and 2C19) inhibited the formation of 18-HC by about 70%. DDC, a purported CYP2E1 inhibitor, was also shown to inhibit 18-HC formation by approximately 25 and 50% at concentrations of K_i and $10K_i$, respectively. However, DDC has been shown to lack selectivity for CYP2E1 (Eagling et al., 1998; Sai et al., 2000). However, a more selective CYP2E1 inhibitor is currently not available (e.g., diallyldisulfide) (Bourrie et al., 1996).

In immunoinhibition studies, CYP2C-directed antibodies were shown to inhibit 18-HC formation by a maximum of 70% in a concentration-dependent manner. CYP2A6-directed antibodies also inhibited 18-HC formation, although to a lesser degree, by a maximum of 30%, at which it appeared to start plateauing. These data suggest an involvement of CYP2A6 in 18-MC *O*-demethylation, although to a lesser extent than CYP2C19. However, the lack of chemical inhibition of 18-HC formation with CYP2A6-selective pilocarpine, combined with the lack of 18-HC formation by microsomes expressing CYP2A6, and the lack of correlation between 18-HC production and CYP2A6 probe activity in a panel of human livers (data not shown), indicates overall that CYP2A6 is not highly involved in 18-HC formation in vitro. The modest CYP2A6 immunoinhibition observed may be due to cross-reactivity. Although a variety of in vitro techniques point to CYP2C19 as the sole high affinity 18-MC *O*-demethylase, the role of CYP2C19 in this pathway in vivo, and the importance of the *O*-demethylation pathway in relation to total clearance, require in vivo studies.

These data characterizing the pathways of 18-MC metabolism (consistent with the dose, 40 mg/kg, used in animal studies) suggest that the 18-MC may be a selective probe substrate of CYP2C19, perhaps more selective than probe substrates used currently (e.g., omeprazole and mephenytoin). Mephenytoin has low affinity for CYP2C19 (K_m values ranging from 59–143 μ M) (Jurima et al., 1985), and its CYP2C19-dependent metabolic ratio has been shown to change with long-term storage of samples (Zhang et al., 1991; Tamminga et al., 2001), whereas the formation of 5-hydroxyomeprazole from omeprazole has been shown to be mediated by CYP2C19 as well as CYP3A4 (Andersson et al., 1993). In vivo studies will be needed to determine whether 18-MC can be used as an in vivo CYP2C19 probe substrate.

CYP2C19 is genetically polymorphic in humans. At least eight defects in the CYP2C19 gene (e.g., 2C19*2, 2C19*3, and 2C19*4) have been discovered (de Morais et al., 1994a,b; Goldstein and de Morais, 1994; Ferguson et al., 1998; see <http://www.imm.ki.se/CYPalleles/cyp2c19.htm>). The importance of CYP2C19 in 18-MC metabolism has several important implications for the clinical pharmacology of the agent itself, although the pharmacological activity and/or toxicity of the major metabolite 18-HC are not clear at present.

For example, CYP2C19 PMs would be expected to have a lower clearance of 18-MC, especially after oral administration, than extensive metabolizers and may thus have altered efficacy and/or toxicity profiles.

Subclinical liver dysfunction or liver disease, particularly cirrhosis, may also affect CYP2C19 activity (Breimer et al., 1975; Zilly et al., 1978). Consequently, patients with liver disease may be at greater risk of toxicity to subtherapeutic doses of the drug. In addition, the potential patient population that would benefit from the therapeutic effects of 18-MC is likely to have taken other medications (prescription or illicit), which may interact with CYP2C19, thus putting them at risk for drug interactions.

In summary, two major conclusions can be drawn from this study. First, the major metabolite of 18-MC in human liver microsomes, 18-HC, is primarily formed by the polymorphic enzyme CYP2C19. Secondly, 18-MC may prove to be a selective and specific probe of CYP2C19 activity in vivo.

References

- Alper KR, Lotsof HS, Frenken GM, Luciano DJ, and Bastiaans J (1999) Treatment of acute opioid withdrawal with ibogaine. *Am J Addict* 8:234–242.
- Andersson TL, Agerstrom PO, Miners JO, Veronesi ME, Weidolf L, and Birkett DJ (1993) High performance liquid chromatographic assay for human liver microsomal omeprazole metabolism. *J Chromatogr* 619:291–297.
- Breimer DD, Zilly W, and Richter E (1975) Pharmacokinetics of hexobarbital in acute hepatitis and after apparent recovery. *Clin Pharmacol Ther* 18:433–440.
- Bertilsson L, Lou Y, Du YL, Liu Y, Kuang TY, Liao XM, Wang KY, Reveiriego J, Iselius L, and Sjoqvist F (1992) Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. *Clin Pharmacol Ther* 51:388–397.
- Bourrie M, Meunier V, Berger Y, and Fabre G (1996) Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. *J Pharmacol Exp Ther* 277:321–332.
- Cappendijk WG and Dzoljic MR (1993) Inhibitory effects of ibogaine on cocaine self-administration in rats. *Eur J Pharmacol* 241:261–265.
- de Morais S, Blaisdell J, Meyer UA, Nakamura K, and Goldstein J (1994a) Identification of a new genetic defect responsible for the polymorphism of S-mephenytoin metabolism in Japanese. *Mol Pharmacol* 46:594–598.
- de Morais S, Blaisdell J, Nakamura K, Meyer UA, and Goldstein J (1994b) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 269:15419–15422.
- Dzoljic ED, Kaplan CD, and Dzoljic MR (1988) Effect of ibogaine on naloxone precipitated withdrawal syndrome in chronic morphine dependent rats. *Arch Int Pharmacodyn Ther* 294:64–70.
- Eagling VA, Tjia JF, and Back DJ (1998) Differential selectivity of cytochrome P450 inhibitors against probe substrates in human and rat liver microsomes. *Br J Clin Pharmacol* 45:107–114.
- Ferguson RJ, Benhamou S, Bouchardy C, Blaisdell J, Ibeanu G, Wilkinson RG, Sarich CT, Wright MJ, Dayer P, and Goldstein AJ (1998) A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of S-mephenytoin. *J Pharmacol Exp Ther* 284:356–361.
- Glick SD, Kuehne ME, Maisonneuve IM, Bandarage UK, and Molinari HH (1996) 18-Methoxycoronaridine, a non-toxic iboga alkaloid congener: effects on morphine and cocaine self-administration and on mesolimbic dopamine release in rat. *Brain Res* 719:39–35.
- Glick SD, Kuehne ME, Raucci J, Wilson TE, Larson TD, Keller RW, and Carlson JN (1994) Effects of iboga alkaloids on morphine and cocaine self-administration in rats: relationship to effects and to effects on dopamine release in nucleus accumbens and striatum. *Brain Res* 657:14–22.
- Glick SD, Maisonneuve IM, Hough LB, Kuehne ME, and Bandarage UK (1999) (±)18-Methoxycoronaridine: a novel iboga alkaloid congener having potential anti-addictive efficacy. *CNS Drug Rev* 5:27–42.
- Glick SD, Maisonneuve LM, Visker KE, Fritz KA, Bandarage UK, and Kuehne ME (1998) 18-Methoxycoronaridine attenuates nicotine-induced dopamine release and nicotine preferences in rats. *Psychopharmacology* 139:274–280.
- Glick SD, Rossman K, Rao NC, Maisonneuve IM, and Carlson JN (1992) Effects of ibogaine on acute signs of morphine withdrawal in rats: independence from tremor. *Neuropharmacology* 31:497–500.
- Glick SD, Rossman K, Steindorf S, Maisonneuve IM, and Carlson JN (1991) Effects and aftereffects of ibogaine on morphine self-administration in rats. *Eur J Pharmacol* 195:341–345.
- Goldstein JA and de Morais SM (1994) Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* 4:285–299.
- Horai Y, Ishizaki T, Ishikawa K, Zhou HH, Zhou BJ, Liao CL, and Zhang LM (1989) Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese versus mainland Chinese. *Clin Pharmacol Ther* 46:198–204.
- Inaba T, Jurima M, Nakano M, and Kalow W (1984) Mephenytoin and sparteine pharmacogenetics in Canadian Caucasians. *Clin Pharmacol Ther* 30:670–676.
- Jurima M, Inaba T, Kadar D, and Kalow W (1985) Genetic polymorphisms of mephenytoin p-(4')-hydroxylation: differences between Orientals and Caucasians. *Br J Clin Pharmacol* 19:483–487.
- Maisonneuve IM and Glick SD (1999) Attenuation of the reinforcing efficacy of morphine by 18-methoxycoronaridine. *Eur J Pharmacol* 383:15–21.
- Mash DC, Kovara CA, Pablo J, Tyndale RF, Ervin FD, Williams IC, Singleton EG, and Mayor M (2000) Ibogaine complex pharmacokinetics, concerns for safety, and preliminary efficacy measures. *Ann NY Acad Sci* 914:394–401.
- Michalek EL (1998) Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* 18:84–112.
- Nakamura K, Ray WA, McAllister CB, Jacqz E, and Wilkinson GR (1985) Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Chinese populations. *Clin Pharmacol Ther* 38:402–408.
- O'Hearn E and Molliver ME (1993) Degeneration of purkinje cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline. *Neuroscience* 55:303–310.
- Sai Y, Dai R, Yang TJ, Krausz KW, Gonzalez FJ, Gelboin FJ, and Shou M (2000) Assessment of specificity of eight chemical inhibitors using cDNA-expressed cytochromes P450. *Xenobiotica* 30:327–343.
- Sershen H, Hashim A, and Lajtha A (1994) Ibogaine reduces preference for cocaine consumption in C57BL/6 mice. *Pharmacol Biochem Behav* 47:13–19.
- Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 270:414–423.
- Tamminga WJ, Wemer J, Oosterhuis B, Wieling J, Touw DJ, de Zeeuw RA, de Leij LF, and Jonkman JH (2001) Mephenytoin as a probe for CYP2C19 phenotyping: effect of sample storage, intra-individual reproducibility and occurrence of adverse events. *Br J Clin Pharmacol* 51:471–474.
- Wedlund PJ, McAllister CB, Wilkinson GR, and Branch RA (1984) Mephenytoin hydroxylation deficiency in Caucasians: frequency of a new oxidative drug metabolism polymorphism. *Clin Pharmacol Ther* 36:773–780.
- Zhang Y, Blouin RA, McNamara PJ, Steinmetz J, and Wedlund PJ (1991) Limitation to the use of the urinary S/R-mephenytoin ratio in pharmacogenetic studies. *Br J Clin Pharmacol* 31:350–352.
- Zilly W, Breimer DD, and Richter E (1978) Hexobarbital disposition in compensated and decompensated cirrhosis of the liver. *Clin Pharmacol Ther* 23:525–534.